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(54) Title: COMPOSITION AND METHOD TO PREVENT GRAFT REJECTION AND OTHER COUNTER–ADAPTIVE T LYMPHOCYTE MEDIATED IMMUNE RESPONSES

(57) Abstract

A method is provided for preventing and reversing acute allograft rejection wherein both the CD80/CD86:CD28/CTLA–4 interaction and the CD40:CD154 interaction are interrupted. The effect of the method on treating autoimmune diseases and allergy is also set forth.

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# COMPOSITION AND METHOD TO PREVENT GRAFT REJECTION AND OTHER COUNTER-ADAPTIVE T LYMPHOCYTE MEDIATED IMMUNE RESPONSES

## FIELD OF THE INVENTION

This invention relates to the field of tissue transplantation, and more particularly to the use of monoclonal antibodies specific for T cell determinants in blocking cell mediated immune responses resulting in allograft or xeongraft rejection.

This invention further relates to the prevention or reversal of graft organ rejection and other counter-adaptive T lymphocyte mediated immune responses. The invention provides compositions and an order and method of treatment to reduce or prevent the rejection of graft organs in primates or man, and to prevent disease resulting from a poorly targeted T lymphocyte mediated immune response.

## BACKGROUND OF THE INVENTION

Organ transplantation between genetically non-identical individuals invariably results in immunological rejection of the organ through T cell dependent mechanisms unless that rejection process is bridled by administering drugs that suppress T cell function. Both calcineurin phosphatase inhibitors and glucocorticosteroids are used clinically, and both prevent the T cell mediated release of activating cytokines, particularly IL-2. Therapy with these agents is imperfect however. Both act by impairing signaling through the T cell antigen receptor (TCR), the sole mediator of T cell antigen specificity, and act on all T cells indiscriminately. In addition, the effect of these drugs is not lasting such that cessation of immunosuppression has generally resulted

in graft loss even after prolonged rejection free survival. Thus, in order to avoid graft rejection, transplant recipients must suffer the consequences of non-specific immunosuppression. These consequences include an increased risk of infection and malignancy as well as significant drug related expense and toxicity.

Data establishing that T cell activation requires both TCR mediated signals and simultaneously delivered costimulatory signals have accumulated over the past 20 years [1]. These important costimulatory signals are provided at least in part by the T cell based CD28 molecule when bound to its counter receptors CD80(B7-1) or CD86 (B7-2), hereafter referred to collectively as B7, on antigen presenting cells (APCs) and perhaps parenchymal cells [1,2,3]. The interaction of CD40 and its T cell based ligand, CD40L (CD154), also plays an important role in T cell activation at least in part by up-regulating B7 [4,5]. In addition, CD40 and CD154 play a fundamental role in establishing T dependent B cell activity [6,7]. Further studies have shown that the T cell molecule CTLA4 (CD152), appears to down-regulate costimulation and TCR mediated activation, at least in part by competing with CD28 for B7 and by delivering a unique negative signal to the TCR signal transduction complex [8].

Several groups have shown in rodents that T cell activation can be blocked and rodent allograft survival prolonged by interfering with B7 interacting with its T cell counter-receptors CD28 and CTLA4 utilizing the B7 specific fusion protein CTLA4-Ig [9-11]. Others have demonstrated that B7 up-regulation can be prevented by the CD154 specific monoclonal antibody MRI[4]. As both agents appear to be dependent on TCR engagement for their effectiveness, the specificity of the T cell response can theoretically be exploited rather than depending on pan T call suppression. In addition to in vitro efficacy, these agents have shown dramatic in

vivo effects in rodents, allowing for the acceptance of fully mismatched skin grafts, a result not obtainable with currently available immunosuppression [12]. It is noteworthy however that all previously reported techniques allowing long-term graft survival in rodents have failed to work or have been associated with major toxicity when tested in species higher on the phylogenetic tree.

## SUMMARY OF THE INVENTION

Accordingly, an object of this invention is a combination of drugs to prevent rejection of transplanted cells, tissues, or organs from either an allogeneic or a xenogeneic source by administering agents that interfere with T cell costimulatory signaling via CD28 when given in conjunction with agents that interfere with the CD40:CD154 interaction.

Another object is a method of treatment to reverse ongoing organ rejection by administering agents that interfere with T cell costimulatory signaling via CD28 when given in conjunction with agents that interfere with the CD40:CD154 interaction.

A third object recognizes that reversal of an ongoing rejection process can be stopped by administering agents that interfere with T cell costimulatory signaling via CD28 when given in conjunction with agents that interfere with the CD40:CD154 interaction.

A fourth object is that for patients currently being treated with standard immunosuppressive therapies (e.g. glucocorticoids, calcineurin phosphatase inhibitors, mycophenolate mofetil) to prevent the rejection of a transplant or to prevent graft versus host disease, those toxic and expensive medications could be discontinued and replaced with short course therapy with agents that interfere with T cell costimulatory signaling via CD28 when given in

conjunction with agents that interfere with the CD40:CD154 interaction.

A fifth object is that for patients with a transplanted organ undergoing chronic rejection, agents that interfere with T cell costimulatory signaling via CD28 when given in conjunction with agents that interfere with the CD40:CD154 interaction can block this undesired immune reaction.

A sixth and most general object is to prevent and/or treat disease states resulting from a counter-adaptive immune response such as the various T-lymphocyte mediated autoimmune illnesses (e.g. insulin dependent diabetes mellitus, multiple sclerosis, etc.) and the various allergic disease states (e.g. hay fever).

A seventh object is to test the hypothesis that CTLA4-Ig and the anti-human CD154 specific monoclonal antibody are capable of inducing tolerance to allografted or even xenografted tissues in humans, and in a more general sense to ameliorate (prevent or treat) all counter-adaptive T-lymphocyte mediated disease states.

These and additional objects of the invention are accomplished by:

1. Utilizing agents that interfere with the interaction of the CD28 and/or CD152 (CTLA4) with their B7 family ligands (CD80 and/or CD86) and with agents that interfere with the interaction of CD40 and CD154 (CD40L). These agents will be administered parenterally (intramuscularly, subcutaneously, or most preferably intravenously) in a standard pharmaceutical carrier (i.e. iv infusion with saline, water, or other buffer).

2. Agents will be administered after cells, tissue(s), or organ(s) have been transplanted. Initial dosing will be administered as soon as the graft is

transplanted at a dose of between 5-20 mg/kg body weight (each agent). Doses will then be administered on days 2,4,6,8,12,16, and 28 post transplant. Thereafter, should signs of immune rejection ensue, dosing will be repeated to reverse the rejection episode. During this retreatment, dosing will be administered as per the initial induction therapy post transplant.

3. This therapy employing agents that interfere with the interaction of both CD28/CD152:B7 and CD40:CD154 will also be administered to individuals with signs indicating that they are developing a disease (including chronic rejection), or that are already suffering with an illness, mediated completely or in part by activated T cells (including patients with a transplant currently receiving standard immunosuppressive therapy). Such "counter-adaptive" T cell responses also include diseases like the various autoimmune illnesses (for example insulin dependent diabetes mellitus, rheumatoid arthritis, multiple sclerosis, inflammatory bowel disease, and systemic lupus erythematosus) as well as in states resulting from the sequela of an immune response like allergic illnesses (hay fever). For these indications, the therapy will be administered in doses ranging from 2-20 mg/kg body weight (each agent) as frequently as every other day for up to 28 days.

4. The "treatment package" will be termed "immune re-education" and will consist of the drugs to be administered, the carrier solvent for those agents, and the infusion system to be used to administer the agent.

This hypothesis is tested in a relevant pre-clinical model. CTLA4-Ig and anti-CD154 were tested alone and in combination on rhesus peripheral blood

leukocytes in vitro, and in rhesus monkeys transplanted with primarily vascularized renal allografts.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1. The effect of CTLA4-Ig and humanized anti-human CD154 alone and in combination on unidirectional rhesus monkey mixed lymphocyte reactions. Increasing concentrations of CTLA4-Ig result in progressive suppression while the effects of humanized anti-human CD154 are more modest. The combination is more effective than either drug alone at 100 fold greater concentrations. Results shown were reproduced in three independent experiments. C.P.M. = counts per minute from incorporated $^{3}H$-thymidine.

Fig. 2 (A) Survival and renal function as determined by serum creatinine following unmodified allogeneic renal transplantation (dashes) or transplantation following induction with CTLA4-Ig alone (squares) or humanized anti-human CD154 alone (diamonds). Open arrows indicate retreatment during biopsy proven rejection. Solid arrows continued survival. (B) Survival and renal function as determined by serum creatinine following unmodified allogenic renal transplantation (dashes) or transplantation following induction with both CTLA4-Ig and humanized anti-human CD154. Open circles indicate treatment on days 0,2,4,6,8,10, and 12 post-transplant. Closed circles indicate treatment on days 0,2,4,6,8,12,16, and 28 post-transplant. Open arrows indicate retreatment during biopsy proven rejection for the animal depicted in open circles. Solid arrows indicate continued survival free of rejection since transplantation.

Fig. 3. (A) Renal allograft histology showing acute cellular rejection following unmodified renal allotransplantation in rhesus monkeys. (B) Renal

allograft histology showing acute cellular rejection prior to reversal with humanized anti-human CD154. (C) Normal renal allograft histology from an animal with normal renal function 163 days following transplantation and induction with CTLA4-Ig and humanized anti-human CD154. (D) A perivascular lymphoid aggregate with the allograft shown in C. These nests of lymphocytes exist in the allograft despite normal function and the absence of immunosuppression. All micrographs are 250x.

Fig. 4. Mixed lymphocyte responses against donor lymphocytes and third party lymphocytes for two rhesus monkeys 150 days after allotransplantation with rejection free survival and normal renal function and without any chronic therapy. Both donor and third party responsiveness is maintained. On the other hand, in data NOT shown, skin grafts placed on a rhesus monkey 6 months following successful allotransplantation revealed donor specific tolerance. Three skin grafts were placed: one from the host (an autograft to control for surgical technique), one from the allogeneic kidney donor, and one from a third party donor. Only the third party donor skin was rejected at day 14 (and counting) since the grafting. This data indicates that functional donor specific tolerance has been achieved despite failure of the allo-MLR to reflect it.

A more complete appreciation of the invention will be readily obtained by reference to the following Description of the Preferred Embodiments and the accompanying drawings in which like numerals in different figures represent the same structures or elements. The representations in each of the figures is diagrammatic and no attempt is made to indicate actual scales or precise ratios. Proportional relationships are shown as approximations.

DESCRIPTION OF PREFERRED EMBODIMENTS

This invention is applicable to both xeno- and allo- transplants, and for more general application to disease states resulting from counter-adaptive T-lymphocyte responses. The invention comprises a composition involving the parenteral administration for an agent interfering with the T cell costimulatory receptors' (CD28/CD152) ability to bind with B7 in close time sequence to administration of an agent preventing signaling through CD152.

The best mode now known initial experience in primates with a new class of reagents directed at modifying T cell costimulation, rather than focused on T cell suppression or elimination. Herein strategies designed to interfere with the interaction of B7 and its counter-receptors CD28 and/or CD152, or with the up regulation of B7 expression are shown to have dramatic effects on T cell responsiveness in vitro, and on allograft survival in vivo- including prevention of rejection and the reversal of established, biopsy proven rejection. In addition, these data demonstrate that anti-rejection activity can persist long after drug administration has stopped. Finally, data is presented to indicate that donor-specific tolerance can be achieved.

It is encouraging that this regimen was remarkably simple, involving two agents administered through a standard peripheral intravenous catheter and that it was tolerated so well by the recipients. This is in stark contrast to other regimens used to achieve lasting graft acceptance in primates requiring ionizing radiation, administration of donor derived bone marrow and significant perioperative immunosuppression [15,16]. The animals treated in this study displayed no evidence to T cell activation or the cytokine release typically observed following treatment with antibodies directed at CD3, and prolonged survival has

not carried with it a demonstrable cost in terms of opportunistic infection. In addition, no alterations in peripheral blood hematological parameters were noted during these studies. Long-term survival was achieved without apparent clearing or global reductions in any lymphocyte subset and without loss of in vitro T cell responsiveness. It is therefore unlikely that the observed effect is attributable to T cell destruction following antibody or fusion protein opsonization. The results are striking. Such success is outbred rhesus monkeys suggests that allograft tolerance is an achievable goal in humans using this or a similar therapeutic approach.

The mechanism and relative contribution of each agent remains a matter of speculation at this juncture. The successes of CD154 blockade alone suggest that any basal costimulation signaling is less important in maintaining the rejection response than B7 up-regulation. Indeed, anti-CD154 resulted in impressive rejection free survival when used alone, while CTLA4-Ig's effects were more transient. Given the recent discovery that CD154 is expressed on non-myeloid cells such as vascular endothelium and smooth muscle [17], and that B7-1 can be induced on fibroblasts [3] and hepatocytes [18], non-T cell events may be critical in establishing reactivity against the allograft. By denying the immune system access to significant parenchymal adhesion and costimulatory signals at the time of transplantation, graft recognition and destruction may be prevented. The differences in activation induced by donor parenchyma and activation induced by lymphoid cells could explain the preservation of in vitro reactivity to donor lymphocytes despite normal graft function, and the general poor correlation between MLR reactivity and clinical graft outcome. Nonetheless, the effects of CTLA4-Ig and humanized anti-human CD154 were shown to be synergistic both in vitro and in vivo. Perhaps,

CTLA4-Ig provides insurance against B7 expression that escapes the effects of humanized anti-human CD154. In that instance, considerable time seems to be required to mount an effective acute rejection with the few cells that escape initial blockade.

As this strategy was successful in reversing established, biopsy proven acute rejection, it would appear that the rejection process must be maintained by continuous costimulation, rather than a process that, once set into motion, proceeds unless the effector cells are eliminated or rendered incapable of TCR signaling. Teleologically, the body is best served by inflammation that is easily controlled. Thus, in the absence of direction to attack, retreat may be the tacit order. This suggests that exploitation of the immune system's natural propensity to down-regulate may be more advantageous than pan-suppression.

The rhesus monkey model used in this study has been shown repeatedly to be a rigorous test of immune manipulation - one that is exquisitely sensitive to even minor changes in allograft function or adverse effects on recipient wound healing and immune function [13,15,19]. In addition, it has obvious biological similarity to human renal transplantation. Specifically, genes that encode MHC proteins are well conversed between rhesus monkeys and humans [20-22], and their rejection of vascularized organs closely parallel that seen clinically [13,15,19]. Nevertheless, issues of optimal dosing and treatment time course remain to be resolved. While rodent models have been successful with a single dose of CTLA4-Ig given on post-operative day 2 in combination with donor specific transfusion [9], it is clear that a more aggressive approach is required in primates. Nonetheless, a transient well tolerated treatment that exploits the specificity of the immune system and gives lasting rejection free survival would appear to be nearing clinical applicability.

Having described the invention, the following examples are given to illustrate specific applications of the invention including the best mode now known to perform the invention. These specific examples are not intended to limit the scope of the invention described in this application.

MATERIALS AND METHODS

Reagents

Human CTLA4-Ig and a control fusion protein-IgG1 were prepared as previously described [2] and shipped in solution by Genetics Institute, Cambridge, MA. The anti-CD40 ligand antibody humanized anti-human CD154 was prepared as previously described [6,7] and shipped in solution by Biogen Corporation, Cambridge, MA. The hamster anti-mouse CD28 monoclonal antibody PV-1 (IgG1, clone G62) was purified from hybridoma culture supernatants and used as in isotype control monoclonal antibody.

MHC Typing and Donor-recipient Selection

Donor-recipient combinations and animals chosen for third party cells were selected based on genetic non-identity at both MHC class I and class II. Class I disparity was established by one-dimensional isoelectric focusing as previously described [13]. Class II disparity was established based on the results of unidirectional mixed lymphocyte reactions (MLRs). In addition, the animal's DRB loci were verified to be disparate by denaturing gradient gel electrophoresis and direct sequencing of the second exon of DRB as previously described [14]. Vigorous in vitro T cell responsiveness of the recipient towards the donor was confirmed in vitro for all donor-recipient pairs. The experiments described in this study were conducted according to the principles set forth in the "Guide for the Care and Use of Laboratory Animals" Institute of

Laboratory Animals Resources, National Research Council, DHHS, Pub. No. (NIH) 86-23 (19850).

In Vitro Cellular Analysis

Unidirectional MLRs were performed on all animals prior to transplantation and on rejection free survivors after 100 days. Each animal was tested against all potential donors to establish the highest responder pairs for transplantation. Responder cells ($3 \times 10^5$) were incubated with irradiated stimulator cells ($1 \times 10^5$) at 37°C for 5 days. Cells were pulse-labeled with $^3$H-thymidine and proliferation was monitored by $^3$H-thymidine incorporation. Polyclonal stimulation with Concanavilin A (25 mcg/ml) served as a positive control. A stimulation index was calculated by normalization to self reactivity, which in all cases was near background incorporation. For in vitro dose response studies, CTLA4-Ig or humanized anti-human CD154 was added to the MLR on day 1 at concentrations ranging from 100 mcg/ml to 0.01 mcg/ml. Combined treatments were performed by varying the CTLA4-Ig concentration and holding the humanized anti-human CD154 concentration steady at 50 mcg/ml.

Peripheral blood lymphocyte phenotype analysis was performed prior to transplantation and periodically during and after drug therapy. Assays evaluated 0.2 ml of heparinized whole blood diluted with phosphate buffered saline and 1% fetal calf serum. FITC labeled T11, B1 (Coulter), and FN18 (the generous gift of Dr. David M. Neville, Jr.) monoclonal antibodies were used to assess the percentage of CD2 (T cell/NK cell), CD20 (B cell), and CD3 (T cell) positive cells respectively. Red blood cells were removed from the preparation by ACK lysis buffer (0.15 M $NH_4Cl$, 1.0 mM $KHCO_3$, 0.1 mM $Na_2$ EDTA, pH 7.3) treatment following staining. Cells were subjected to flow cytometry immediately, or following

fixation in 1% paraformaldehyde. Flow cytometry was performed using a Becton Dickinson FACSCAN.

Renal Allografts

Renal allotransplantation was performed as previously described [13]. Briefly, outbred juvenile (1-3 years of age) rhesus monkeys, seronegative for simian immunodeficiency virus, simian retrovirus, and herpes B virus, were obtained from the Primate Center (University of Wisconsin) or LABS (Yemassee, SC). Procedures were performed under general anesthesia using ketamine (1 mg/kg, i.m.), xylazine (1 mg/kg, i.m.) and halothane (1%, inhaled). Transplantation was performed between genetically distinct donor-recipient pairs as determined by the MHC analysis described above. The animals were heparinized during organ harvest and implantation (100 units/kg). The allograft was implanted using standard microvascular techniques to create an end to side anastamosis between the donor renal artery and recipient distal aorta as well as the donor renal vein and recipient vena cava. A primary ureteroneocystostomy was then created. Bilateral native nephrectomy was completed prior to closure.

Animals were treated with intravenous fluid for approximately 36 hours until oral intake was adequate. Trimethaprim-sulfa was administered for 3 days for surgical antibiotic prophylaxis. Each animal received 81 mg of aspirin on the day of surgery. The need for analgesia was assessed frequently and analgesia was maintained with intramuscular butorphanol. Animals were weighed weekly. Skin sutures were removed after 7 to 10 days. CTLA4-Ig and/or humanized anti-human CD154, was given intravenously at doses and dosing schedules varying based on accumulating experience with the agents. No other immunopharmaceuticals were administered. Serum creatinine, and whole blood electrolytes ($Na^+$, $K^+$, $Ca^{2+}$) and hemoglobin were

determined every other day until stable and then weekly.

Pathological Analysis

Biopsies were performed on animals suspected of having rejection using a 20-gauge needle core device (Biopty-Cut, Bard). Standard staining with hematoxylin and eosin was performed on frozen or formalin fixed tissue to confirm the diagnosis of rejection. Animals were euthanized at the time of anuria or if a weight loss of 15% of pre-transplant body weight occurred in accordance with AAALAC standards. All animals underwent complete gross and histopathological evaluation at the time of death.

RESULTS

CTLA4-Ig and humanized anti-human CD154 synergistically prevent T cell proliferation in vitro.

Both CTLA4-Ig and humanized anti-human CD154 inhibited rhesus MLRs in a dose dependent fashion (Fig. 1). CTLA4-Ig was, however, more effective than humanized anti-human CD154 as a single agent in preventing T cell proliferation. Substantial reduction in thymidine incorporation was seen at a CTLA4-Ig concentration of 0.1 mcg/ml, and further inhibition was achieved at higher concentrations. Modest reduction in proliferation was achieved with humanized anti-human CD154 concentrations of 0.01 mcg/ml but inhibition was not substantially improved by increasing concentrations. Both agents acted synergistically, the combination inhibiting proliferation approximately 100 times more effectively than either agent alone did. Dose response studies were repeated for 3 separate naive animals with identical results. CTLA4-Ig and humanized anti-human CD154 synergistically prevent allograft rejection in vivo.

Twelve renal allotransplants were performed (Fig. 2). Four animals received transplants without any immunological intervention. These animals rejected in 5,7,7 and 8 days. Histological examination of their kidneys showed acute cellular rejection characterized by diffuse interstitial and tubular lymphocytic infiltration with edema and cellular necrosis (Fig. 3A). One animal was given a 5-day course of CTLA4-Ig (10 mg/kg/d) beginning at the time of transplantation and had graft survival prolonged to 20 days (Fig. 2A). Graft loss was due to cellular rejection indistinguishable from that seen in the control animals. One animal was treated with CTLA4-Ig 20 mg/kg on the day of transplantation followed by a 12 day course of 10 mg/kg every other day and had graft survival prolonged to 30 days (Fig. 2A). Again, graft loss was due to acute cellular rejection. Extrapolating from previously published work in the rat heterotopic cardiac allograft model of Turka, et al [9] a donor specific transfusion of lymph node derived lymphocytes ($10^8$) was given at the time of transplantation to this 2 animals.

Two animals were treated with humanized anti-human CD154 alone (Fig. 2A). Both animals received 20 mg/kg every other day beginning on the day of surgery and continuing for 14 post-operative days (8 doses total). Both animals experienced extended rejection free survival, although transient creatinine elevations were recorded during the second and fourth post-operative weeks. Both animals rejected between 95 and 100 days post-transplant. Biopsy was performed on each animal to confirm the diagnosis (Fig. 3B). Both animals were then retreated with 7 doses of humanized anti-human CD154 (20 mg/kg; one animal every other day and one animal daily) and both returned to normal graft function with no demonstrable adverse effects. They remain alive and well greater than 150 days after transplantation at the time of this writing.

Two animals were given 20 mg/kg each of CTLA4-Ig and humanized anti-human CD154 following transplantation (Fig. 2B). Again, each drug was given every other day beginning on the day of surgery and continuing for 14 post-operative days. One animal rejected 32 days after surgery. The other remained free of rejection for 100 days, but like those animals treated with humanized anti-human CD154 alone, rejected at that time. Similarly, a biopsy showed acute cellular rejection. The initial regimen of CTLA4-Ig and humanized anti-human CD154 was repeated and the creatinine returned to baseline (1.0). MLR analysis following this treatment showed a donor specific loss of reactivity. Third party responsiveness was maintained. At 165 days post transplant, the animal was sacrificed as required by protocol due to weight loss. Graft function at that time was normal. At autopsy, the animal was found to have shigella and camphylobacter enterocolitis, a common infection in rhesus monkeys. This illness had infected multiple animals in the original primate colony, including several untreated animals. No other pathological abnormality was found; specifically, there was no evidence of lymphoproliferative disease or opportunistic infection. Histologically, the graft had isolated nests of lymphocytes in the interstitium, but no evidence of tubular infiltration, glomerular damage, or parenchymal necrosis (Fig. 3C).

Like the animals treated with humanized anti-human CD154 alone, both of these animals had transient increases in their creatinine combined with an increase in graft size during the fourth post-operative week. It was hypothesized that this graft swelling reflected a second wave of infiltrating lymphocytes and therefore led to a modified dosage schedule such that both reagents were given on the day of surgery and on post-operative days 2,4,6,8, 12, 16, and 28.

Two animals were treated with this modified regimen (Fig. 2B). Both have experienced rejection free survival, free of illness or alterations in renal function for greater than 150 days. Both remain alive and well at the time of this writing. After 100 days of rejection free survival, MLRs were repeated against donor cells and third party cells. No changes in in vitro reactivity were observed (data not shown). These studies were repeated after 150 days of rejection free survival with identical results (Fig. 4). Both animals maintain vigorous in vitro responses toward donor and third party cells but fail to reject their allografts.

No animal has demonstrated toxicity from any of the therapies employed. Specifically, there has been no fever, anorexia, or hemodynamic abnormalities, and no opportunistic infections have occurred. Animals have been housed in standard conditions and have been allowed contact with the other animals in the colony. They have maintained normal weight gain. Laboratory chemistries and hematological parameters such as hemoglobin and white blood cell counts have remained normal. The percentages of cells expressing CD2, CD3 and CD20 were unaffected by any treatment regimen (data not shown). Specifically, no reductions in T cell counts were observed during or after treatment in any animal.

Obviously, many modifications and variations of the present invention are possible in light of the above teachings. It is therefore to be understood that, within the scope of the appended claims, the invention may be practiced otherwise than as specifically described.

CLAIMS

What is claimed is:

Claim 1. A composition for the suppression of organ transplant rejection compromising agents that interrupt B-7(CD80/CD86):CD28/CD152 interaction in combination with agents that interfere with the CD40:CD154 interaction.

Claim 2. The composition of Claim 1 wherein the B-7:CD28 interrupter is selected from the group consisting of CTLA4-Ig, anti CD80 antibody, anti CD86 antibody, anti CD28 antibody, anti CD152 antibody and fragments and modifications of that interrupter and the CD40:CD154 interrupter and fractions and modifications of that interrupter, an anti CD154 antibody.

Claim 3. The composition of Claim 2 comprising approximately 5-20 mg/kg of B-7:CD28 interrupter and 5-20 mg/kg of CD40:CD154 interrupter.

Claims 4. The composition of Claim 1 wherein the agents interfere with the interaction of the CD28 and/or CD152 (CTLA4) with their B7 family ligands (CD80 and/or CD86) and with agents that interfere with the interaction of CD40 and CD154(CD40L).

Claim 5. A treatment regime for suppressing organ transplant rejection and inducing tolerance comprising administering at least 2 doses of a B-7:CD28 interrupter agent in combination with a CD40:CD154 interrupter agent in at least the first 5 days following transplantation replacement of an organ.

Claim 6. The treatment regime of Claim 4 wherein 5-20 mg/kg body weight of each of a B-7:CD28 interrupter agent combination with a CD40:CD154 interrupter agent

is administered up to every 2-4 days for up to the first month following transplantation.

Claim 7. The treatment regime of Claim 5 wherein the combination of agents is administered together.

Claim 8. The treatment regime of Claim 5 wherein the combination of agents are administered separately in the same day.

Claim 9. The treatment regime of Claim 6 wherein the B-7:CD28 interrupter is selected from the group consisting of CTLA4-Ig, anti CD80 antibody, anti CD86 antibody, anti CD28 anitbody, anti CD152 antibody and fragments and modifications of that interrupter and the CD40:CD154 interrupter and fractions and modifications of that interrupter, an anti CD154 antibody.

Claim 10. The treatment of Claim 6 wherein the combination is administered parenterally.

Claim 11. The treatment regime of Claim 6 wherein the combination is administered by the means selected from the group consisting of intramuscularly, subcutaneously, and intravenously in a standard pharmaceutical carrier.

Claim 12. The treatment regime of Claim 5 wherein the treatment is administered to reverse ongoing organ rejection by administering agents that interfere with T cell costimulatory signaling via CD28 when given in conjunction with agents that interfere with the CD40:CD154 interaction.

Claim 13. The treatment regime of Claim 5 wherein the treatment is administered to patients receiving immunosuppressant drugs to wean them from the immunosuppressant drugs.

Claim 14. A treatment of disease states resulting from a counter-adaptive immune response such as the various T-lymphocyte mediated autoimmune illnesses (e.g. insulin dependent diabetes mellitus, multiple sclerosis, etc.) and the various allergic disease states (e.g. hay fever) comprising administering at least 2 doses of a B-7:CD28 interrupter agent in combination with a CD40:CD154 interrupter agent in for at least 5 days.

Claim 15. The treatment of Claim 5 wherein the initial dosing is administered as soon as the graft is transplanted at a dose of between 5-20 mg/kg body weight (each agent) and doses are administered on days 2,4,6,8,12,16, and 28 post transplant.

Claim 16. The treatment of Claim 15 wherein dosing will be repeated to reverse the rejection episode should signs of immune rejection ensue, and during this retreatment, dosing is administered as per the initial induction therapy post transplant.

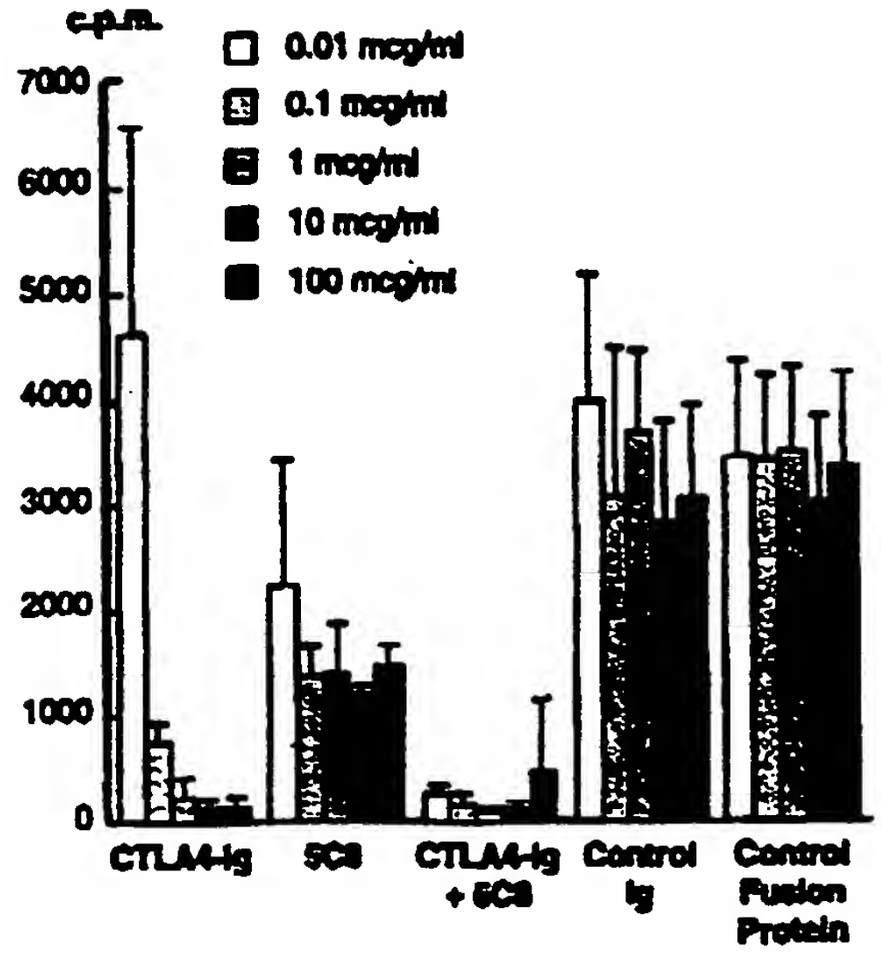
c.p.m.
7000
6000
5000
4000
3000
2000
1000
0
0.01 mcg/ml
0.1 mcg/ml
1 mcg/ml
10 mcg/ml
100 mcg/ml
CTLA4-Ig
5C8
CTLA4-Ig + 5C8
Control Ig
Control Fusion Protein

FIG. 1

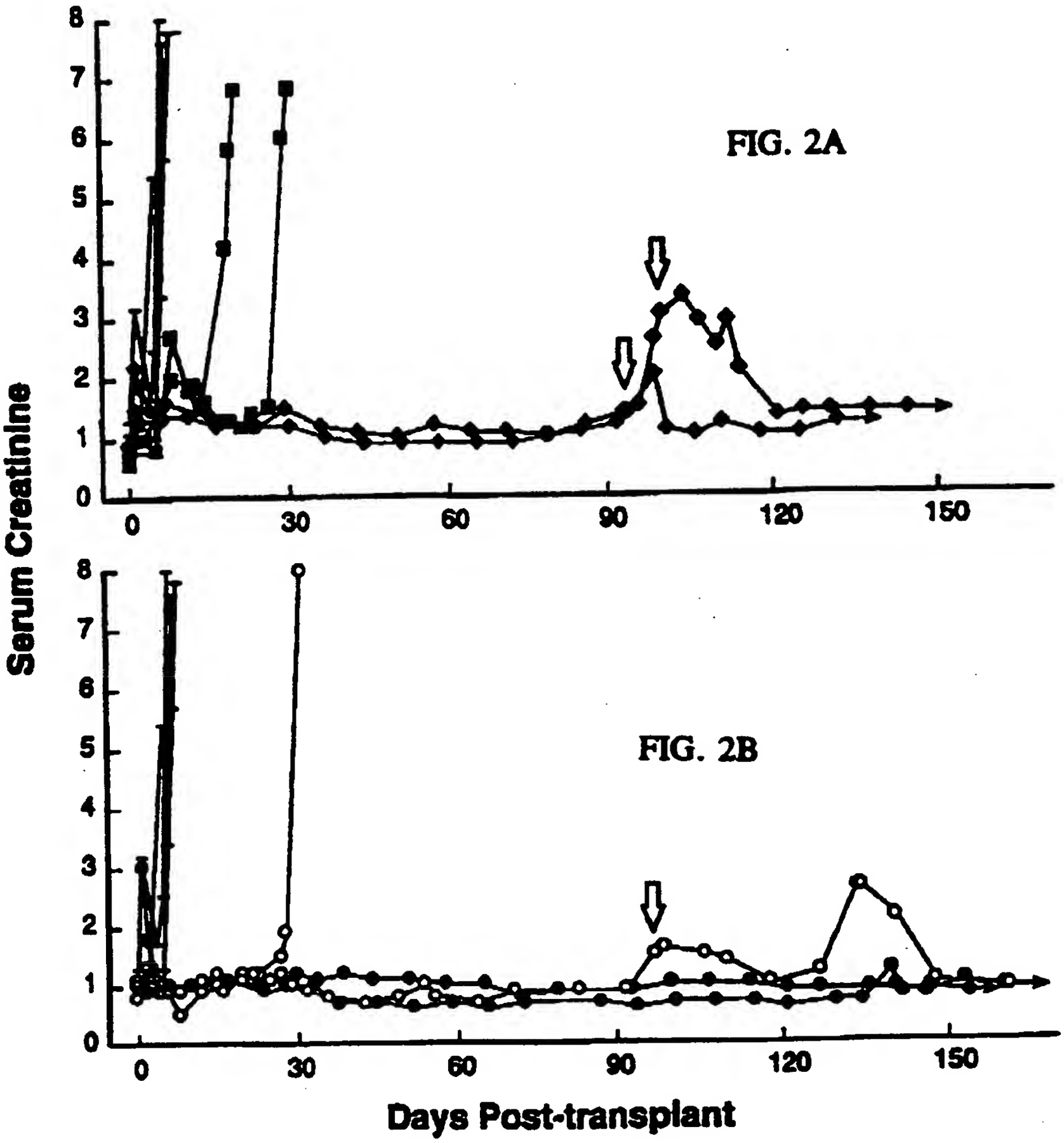
Serum Creatinine
0
1
2
3
4
5
6
7
8
0
30
60
90
120
150
Days Post-transplant

FIG. 2A

FIG. 2B

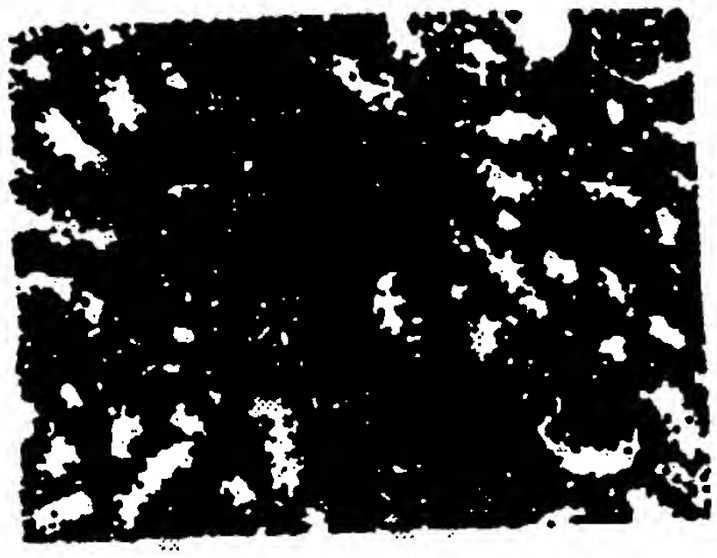

FIG. 3A

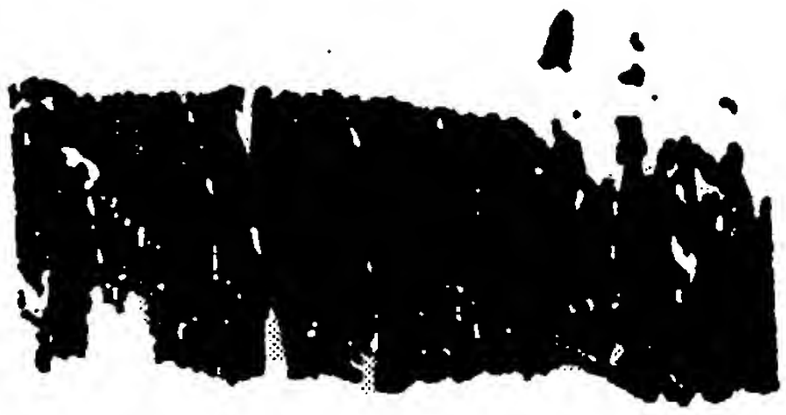

FIG. 3B

FIG. 3C

FIG. 3D

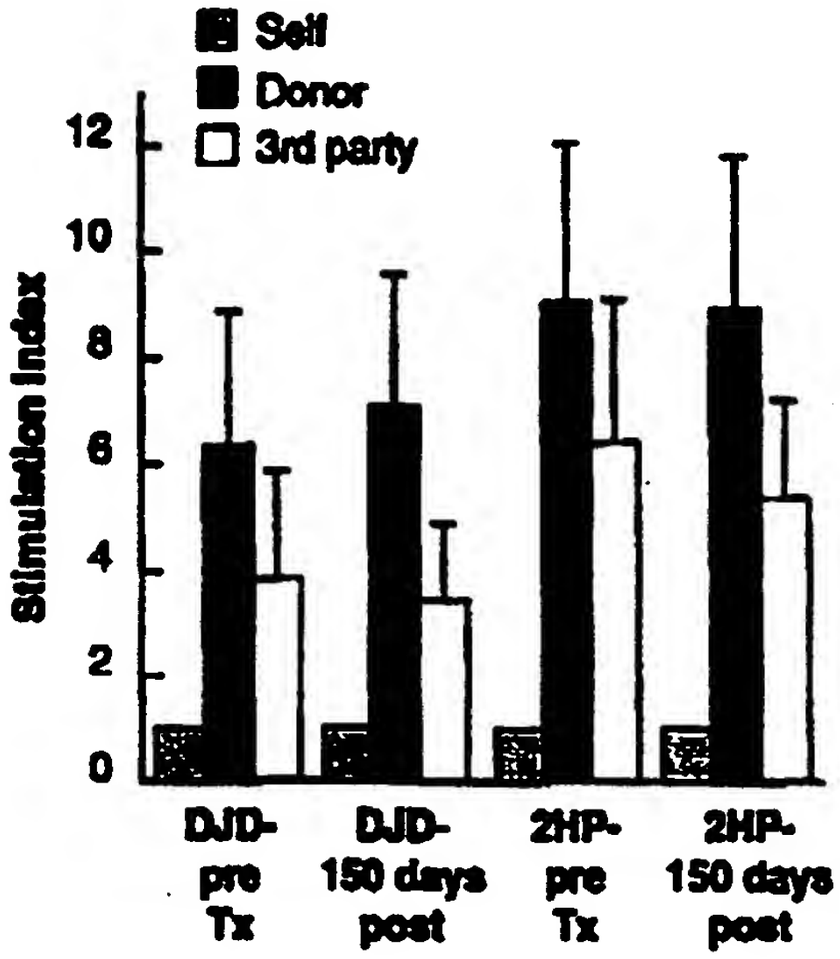
Self
Donor
3rd party
Stimulation Index
12
10
8
6
4
2
0
DJD-pre Tx
DJD-150 days post
2HP-pre Tx
2HP-150 days post

FIG. 4

# INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/11910

A. CLASSIFICATION OF SUBJECT MATTER
IPC(6) :A61K 39/395; C07K 16/28; C12P 21/08
US CL :424/144.1, 154.1, 173.1, 192.1; 530/388.22, 388.75, 389.6
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/144.1, 154.1, 173.1, 192.1; 530/388.22, 388.75, 389.6

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

MEDLINE, BIOSIS, WPIDS, CAPLUS, APS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X | LARSEN et al. Long-term Acceptance of Skin and Cardiac Allografts After Blocking CD40 and CD28 Pathways. Nature. 30 May 1996, Vol. 381, pages 434-438, see entire document. | 1-13, 15, 16 |
| X | ELWOOD et al. Long-term Murine Skin Allograft Survival with Perioperative CTLA4-Ig and Anti-gp39: The Role of CD4+ T Cells. Surgical Forum. 1996, Vol. 47, pages 427-429, see entire document. | 1-13, 15, 16 |
| X | WO 95/34320 A2 (REGENTS OF THE UNIVERSITY OF MINNESOTA) 21 December 1995, see entire document. | 1-16 |

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

| | | | |
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| * | Special categories of cited documents: | "T" | later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention |
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| "E" | earlier document published on or after the international filing date | "Y" | document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art |
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| "O" | document referring to an oral disclosure, use, exhibition or other means | | |
| "P" | document published prior to the international filing date but later than the priority date claimed | | |

| | |
|---|---|
| Date of the actual completion of the international search<br>29 AUGUST 1998 | Date of mailing of the international search report<br>28 SEP 1998 |
| Name and mailing address of the ISA/US<br>Commissioner of Patents and Trademarks<br>Box PCT<br>Washington, D.C. 20231<br>Facsimile No. (703) 305-3230 | Authorized officer<br>EVELYN RABIN<br>Telephone No. (703) 308-0196 |

Form PCT/ISA/210 (second sheet)(July 1992)*